



## **8. Bioconductor Intro**

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# What is Bioconductor?



The image shows the Bioconductor website header and navigation menu. The header features the Bioconductor logo (a stylized 'B' with a DNA helix) and the text "BIOCONDUCTOR open source software for bioinformatics". To the right of the logo is a description: "Bioconductor is an open source and open development software project for the analysis and comprehension of genomic data." Below the header is a navigation menu with buttons for "home", "getting started", "overview", "downloads", "documentation", "publications", "workshops", and "cabig". The main content area is divided into two columns. The left column is titled "QUICK LINKS" and contains a list of links: "Getting Started", "Installation", "Downloads", "Software", "Workshops", "FAQ", and "Mailing Lists". The right column is titled "project news" and contains a news item dated "2009-01-07" about R, the open source platform used by Bioconductor, featured in the New York Times. Below the news item is a "More..." link. At the bottom of the page, there are four conference and course announcements, each with a title, location, date, and a "Details and Registration" link.

**BIOCONDUCTOR**  
open source software for bioinformatics

Bioconductor is an open source and open development software project for the analysis and comprehension of genomic data.

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**project news**

▶ [2009-01-07](#)  
R, the open source platform used by Bioconductor, featured in a series of articles in the New York Times.  
[More...](#)

**QUICK LINKS**

- ▶ [Getting Started](#)
- ▶ [Installation](#)
- ▶ [Downloads](#)
- ▶ [Software](#)
- ▶ [Workshops](#)
- ▶ [FAQ](#)
- ▶ [Mailing Lists](#)

**BioC2009 Conference**  
Seattle, WA, 27-28 July 2009. [🔒 Details and Registration](#)

**CSAMA09 BioConductor Course**  
Brixen, Italy, 14-19 June 2009. [🌐 Details and Registration](#)

**Statistical Microarray Analysis Using R (and Bioconductor)**  
London, England, 18-19 June 2009. [🌐 Details and Registration](#)

**Statistical analysis of gene expression data with R and Bioconductor**  
Copenhagen, Denmark, 17-21 August 2009. [🌐 Details and Registration](#)

# What is Bioconductor?

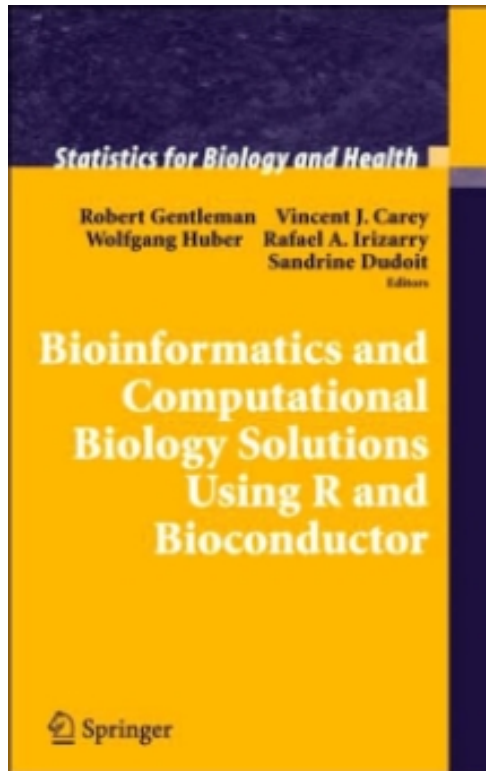
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- `www.bioconductor.org`
- Software project for analysis of genomic data – and related tools, resources/datasets
- **Open source** and **Open development**
- **Free**

You **could** use commercial software; but **experts** typically **write R code first**. The help manuals are **not a sales pitch** and encourage **appropriate** use

# Bioconductor basics

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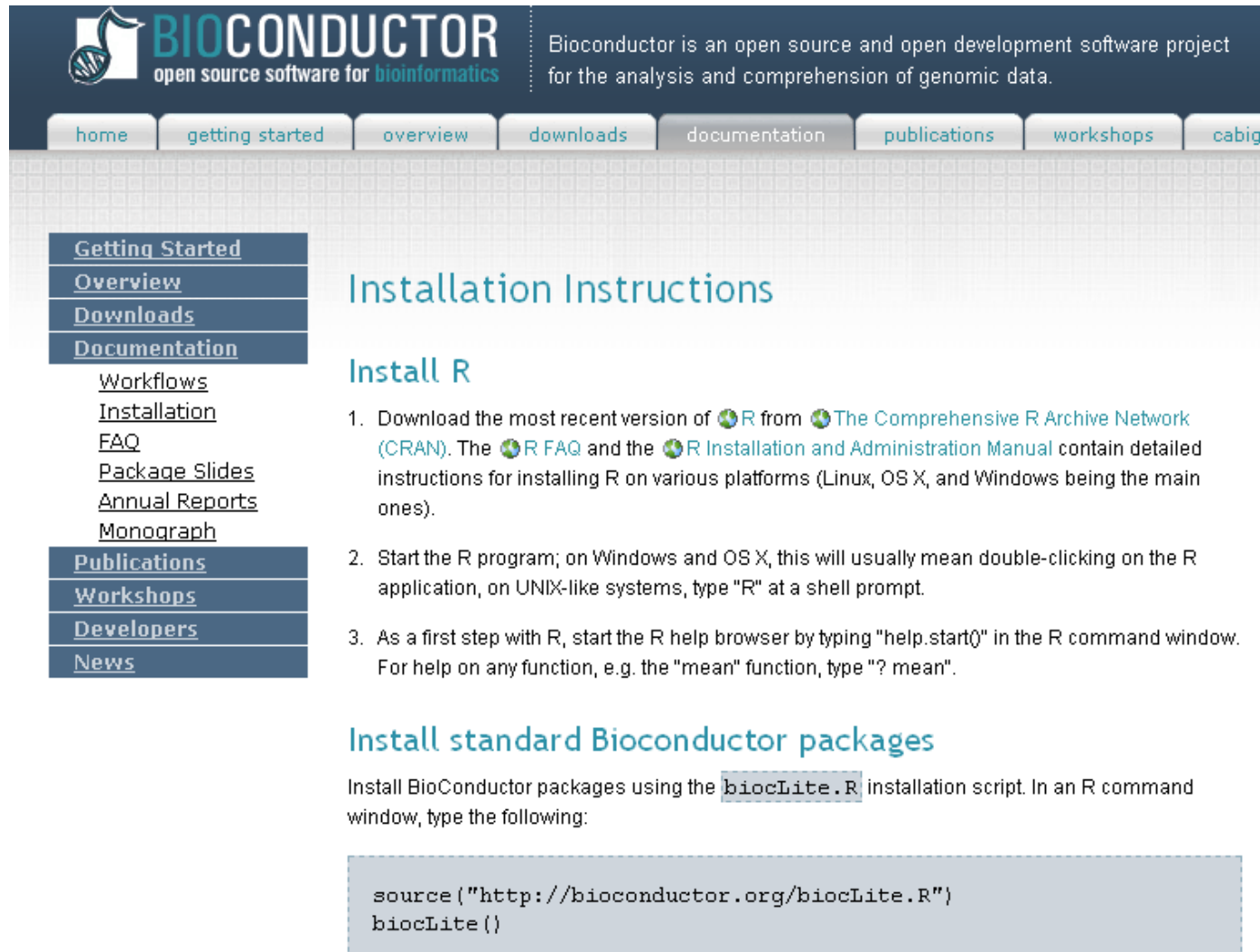
- Begun in 2001, based at Harvard and now FHCRC (Seattle)
- A large collection of R packages (they also convert good software to R)
- Far too much for our little course!

We'll give examples of what Bioconductor can do, and how to learn more. Gentleman et al (above) is a helpful reference text

# Bioconductor basics

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Getting started...



The screenshot shows the Bioconductor website's 'Installation Instructions' page. At the top, the Bioconductor logo is displayed with the tagline 'open source software for bioinformatics'. A navigation bar contains links for 'home', 'getting started', 'overview', 'downloads', 'documentation', 'publications', 'workshops', and 'cabig'. The main content area features a sidebar with a 'Getting Started' menu containing links for 'Overview', 'Downloads', 'Documentation', 'Workflows', 'Installation', 'FAQ', 'Package Slides', 'Annual Reports', and 'Monograph'. Below this are links for 'Publications', 'Workshops', 'Developers', and 'News'. The main heading is 'Installation Instructions', followed by a sub-heading 'Install R'. A three-step list provides instructions on downloading R from CRAN, starting the R program, and starting the R help browser. Below the list is a section titled 'Install standard Bioconductor packages' which explains the use of the 'biocLite.R' script and provides a code block for its execution.

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Getting Started  
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News

## Installation Instructions

### Install R

1. Download the most recent version of [R](#) from [The Comprehensive R Archive Network \(CRAN\)](#). The [R FAQ](#) and the [R Installation and Administration Manual](#) contain detailed instructions for installing R on various platforms (Linux, OS X, and Windows being the main ones).
2. Start the R program; on Windows and OS X, this will usually mean double-clicking on the R application, on UNIX-like systems, type "R" at a shell prompt.
3. As a first step with R, start the R help browser by typing "help.start()" in the R command window. For help on any function, e.g. the "mean" function, type "? mean".

### Install standard Bioconductor packages

Install BioConductor packages using the `biocLite.R` installation script. In an R command window, type the following:

```
source("http://bioconductor.org/biocLite.R")
biocLite()
```

# Bioconductor basics

---

```
> source("http://bioconductor.org/biocLite.R")
> biocLite()
```

installs the following libraries;

```
affy, affydata, affyPLM, annaffy, annotate, Biobase,
Biostrings, DynDoc, gcrma, genefilter, geneplotter, hgu95av2.db,
limma, marray, matchprobes, multtest, ROC, vsn, xtable,
affyQCReport
```

... then you use e.g. `library(ROC)` as before.

`vignette(package="ROC")` tells you to look at `vignette("ROCnotes")` for a **worked example** – a very helpful introduction. (Or use e.g. `openVignette("ROC")` from the Biobase package)

# Bioconductor basics

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To get other packages, use e.g. `biocLite("SNPchip")`

**Do not** need to type `biocLite()` after you install (even in a new R session).

This would install everything again – which is harmless, but slow.

# What to install?

---

Back to the front page – click ‘Downloads’



The screenshot shows the Bioconductor website interface. At the top left is the Bioconductor logo, a stylized DNA double helix with a blue and white color scheme, followed by the text "BIOCONDUCTOR" in large blue letters and "open source software for bioinformatics" in smaller white letters below it. To the right of the logo is a dark blue banner with white text: "Bioconductor is an open source and open development software project for the analysis and comprehension of genomic data." Below the banner is a horizontal navigation bar with buttons for "home", "getting started", "overview", "downloads", "documentation", "publications", "workshops", and "cabig". The "downloads" button is highlighted. On the left side, there is a vertical menu with buttons for "Getting Started", "Overview", "Downloads", "Documentation", "Publications", "Workshops", "Developers", and "News". The "Downloads" button is highlighted. The main content area has a light gray grid background. The heading "BioC 2.4 (release)" is displayed in large blue text. Below it, a bold black text states: "BioC 2.4 was released 21 April, 2008. This release is designed for R 2.9.z." Underneath, the section "BioC 2.4 packages:" is followed by a bulleted list: "Software", "Metadata (Annotation, CDF and Probe)", "Experiment Data", and "Complete Taxonomy". At the bottom of the main content area, there are two links: "Installation Instructions" and "Multiple platform build/check report".

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Getting Started  
Overview  
**Downloads**  
Documentation  
Publications  
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Developers  
News

## BioC 2.4 (release)

**BioC 2.4 was released 21 April, 2008. This release is designed for R 2.9.z.**

**BioC 2.4 packages:**

- Software
- Metadata (Annotation, CDF and Probe).
- Experiment Data
- Complete Taxonomy

[Installation Instructions](#)

[Multiple platform build/check report](#)



# What to install?

---

- **Software** – probably what you want
- **Annotation data** – e.g. probe sequence data for microarrays of different types
- **Experiment data** – e.g. datasets from [hapmap.org](http://hapmap.org), some expression datasets
- **Complete Taxonomy** – just everything!

# Software example – hexbin

---

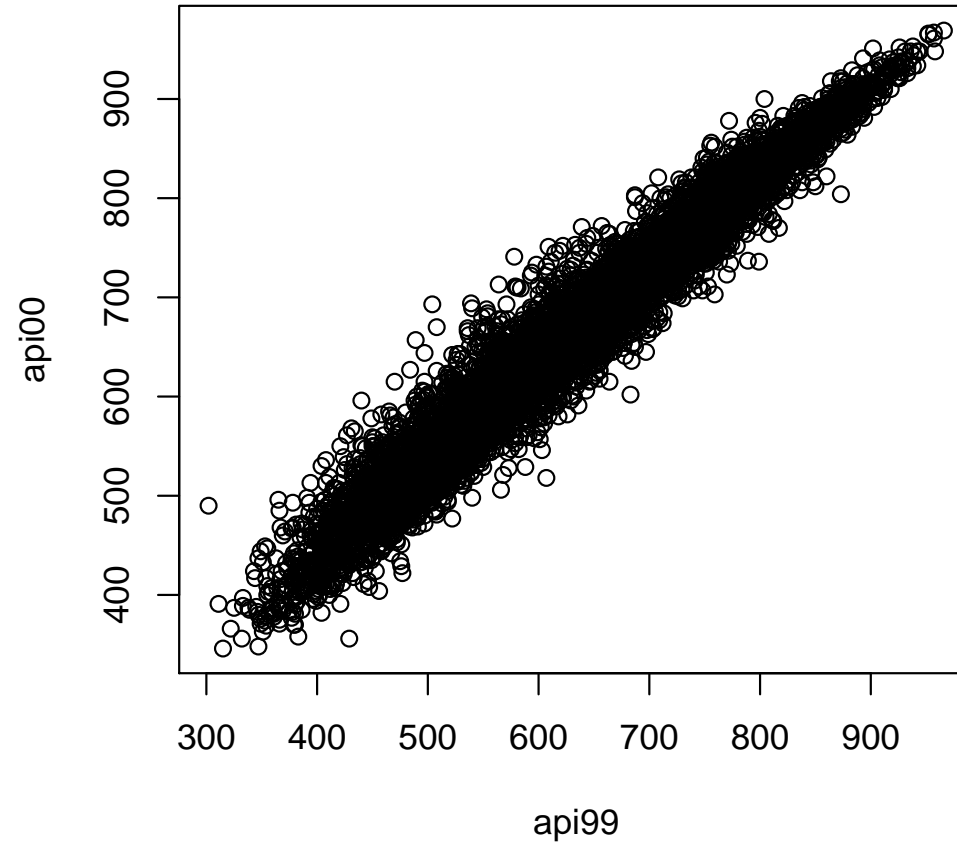
Genetics/Genomics tends to produce **massive** datasets. On any (standard) plot of e.g. 10,000 points, **many** will overlap

Recall the California schools example – the California Academic Performance Index reported from 6194 schools; download the (standard) package

```
> install.packages("survey")
> library(survey)
> data(api)
> plot(api00~api99,data=apipop) # plain plot
```

# Software example – hexbin

---



# Hexbin – a better way

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We don't *really* care about the exact location of every single point.

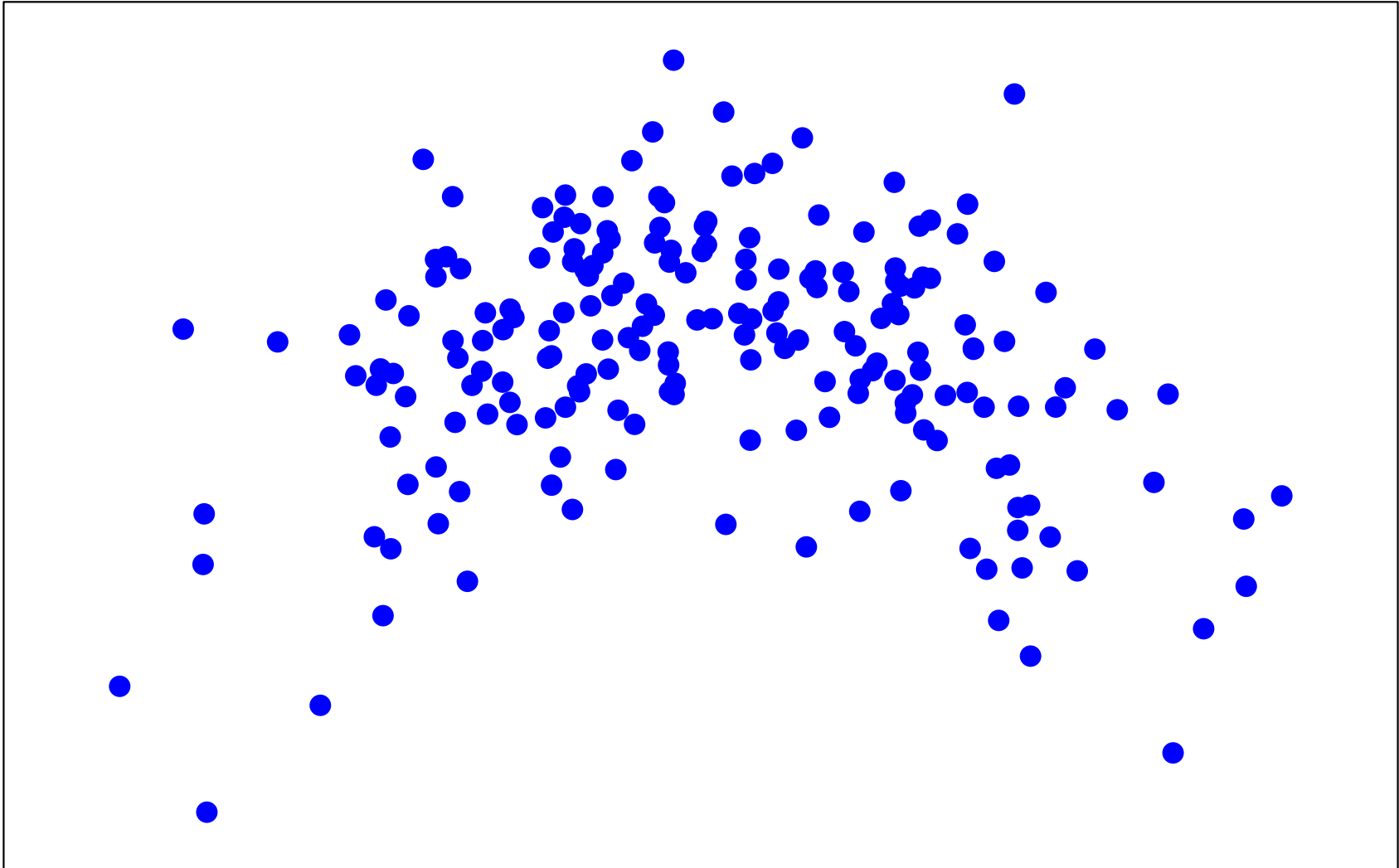
- How **many** points in one 'vicinity' compared to others?
- Any 'outliers' far from all other data points?

In one dimension, histograms answer these questions by **binning** the data

# Hexbin – a better way

---

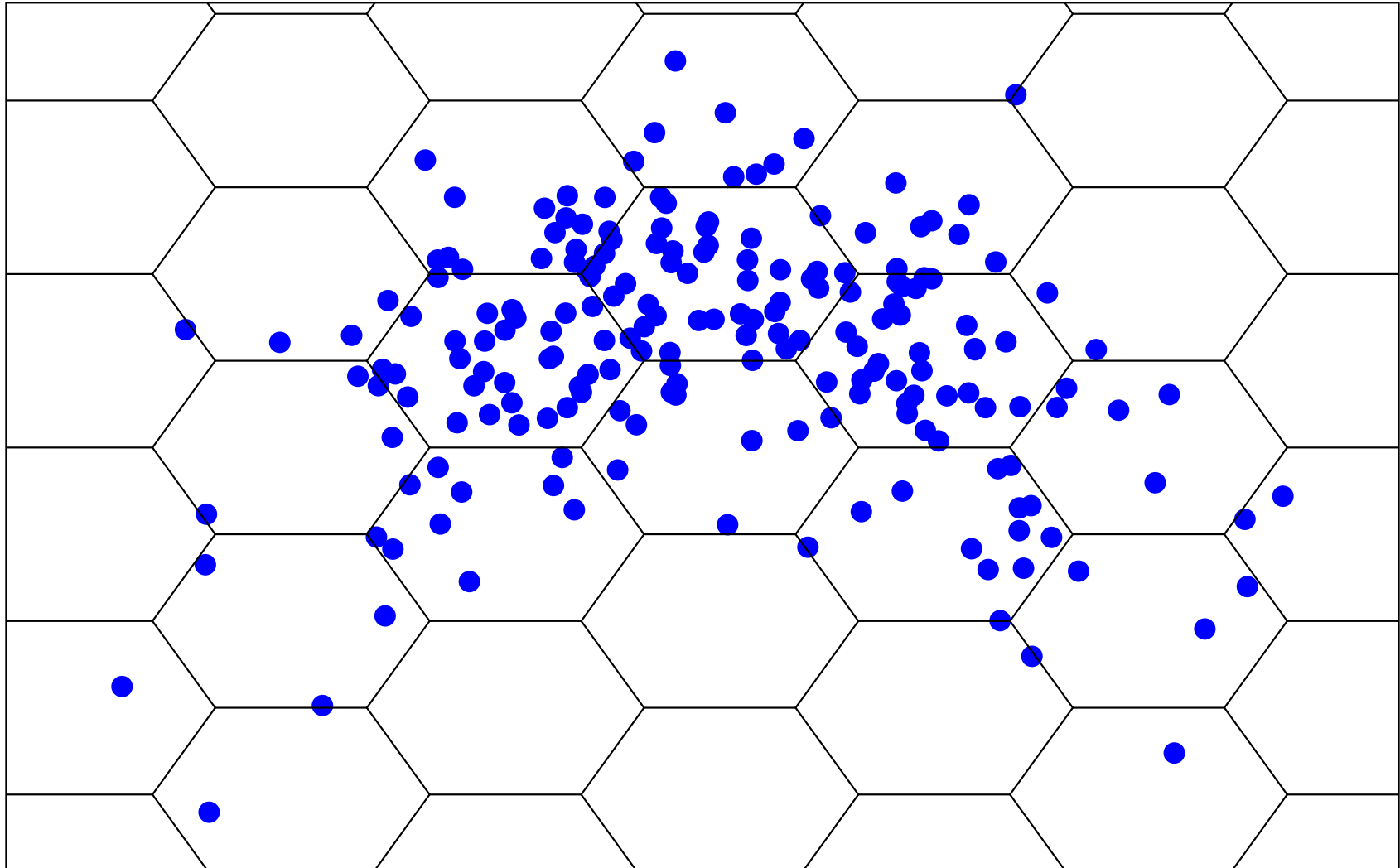
Binning in two dimensions;



# Hexbin – a better way

---

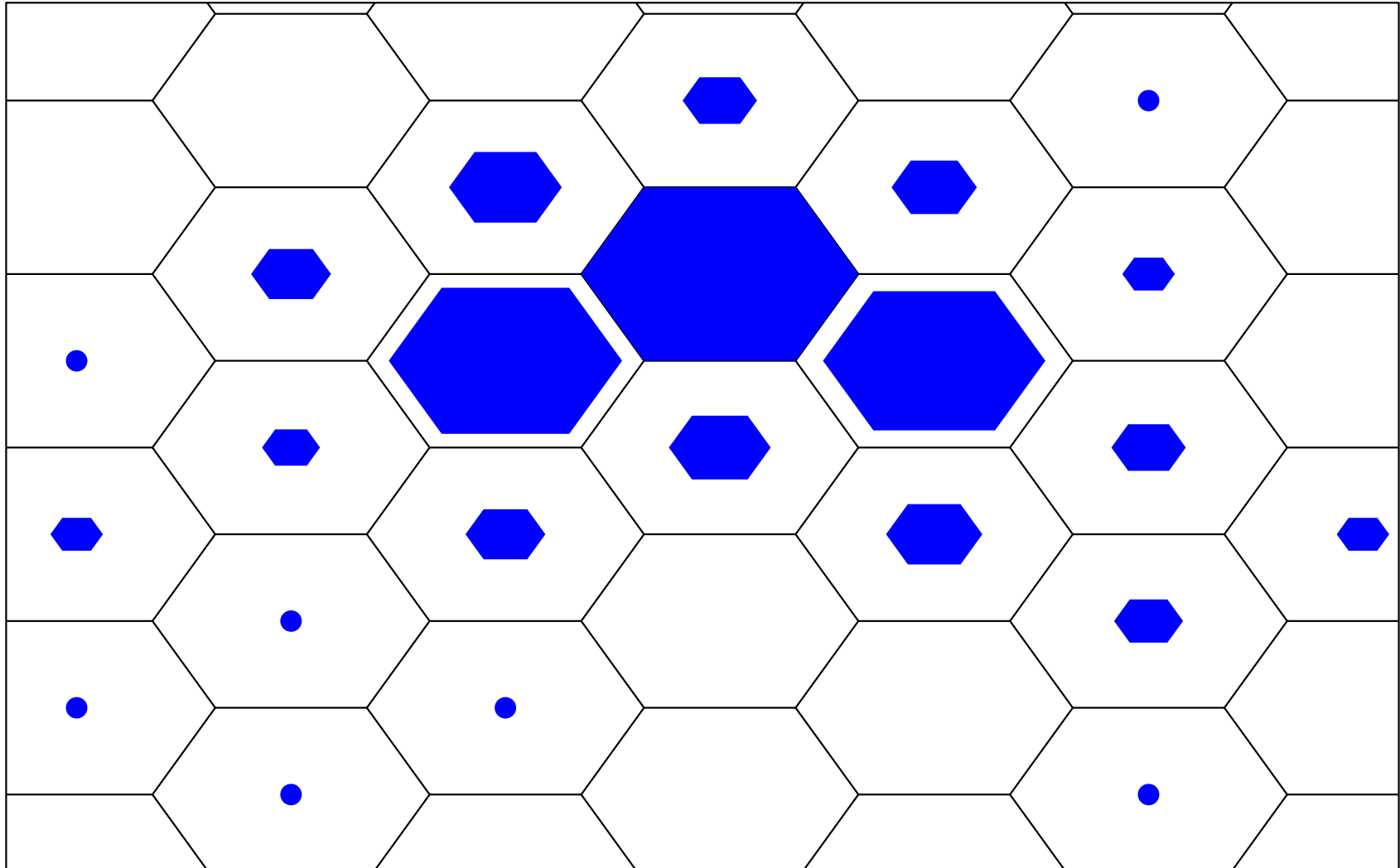
Binning in two dimensions;



# Hexbin – a better way

---

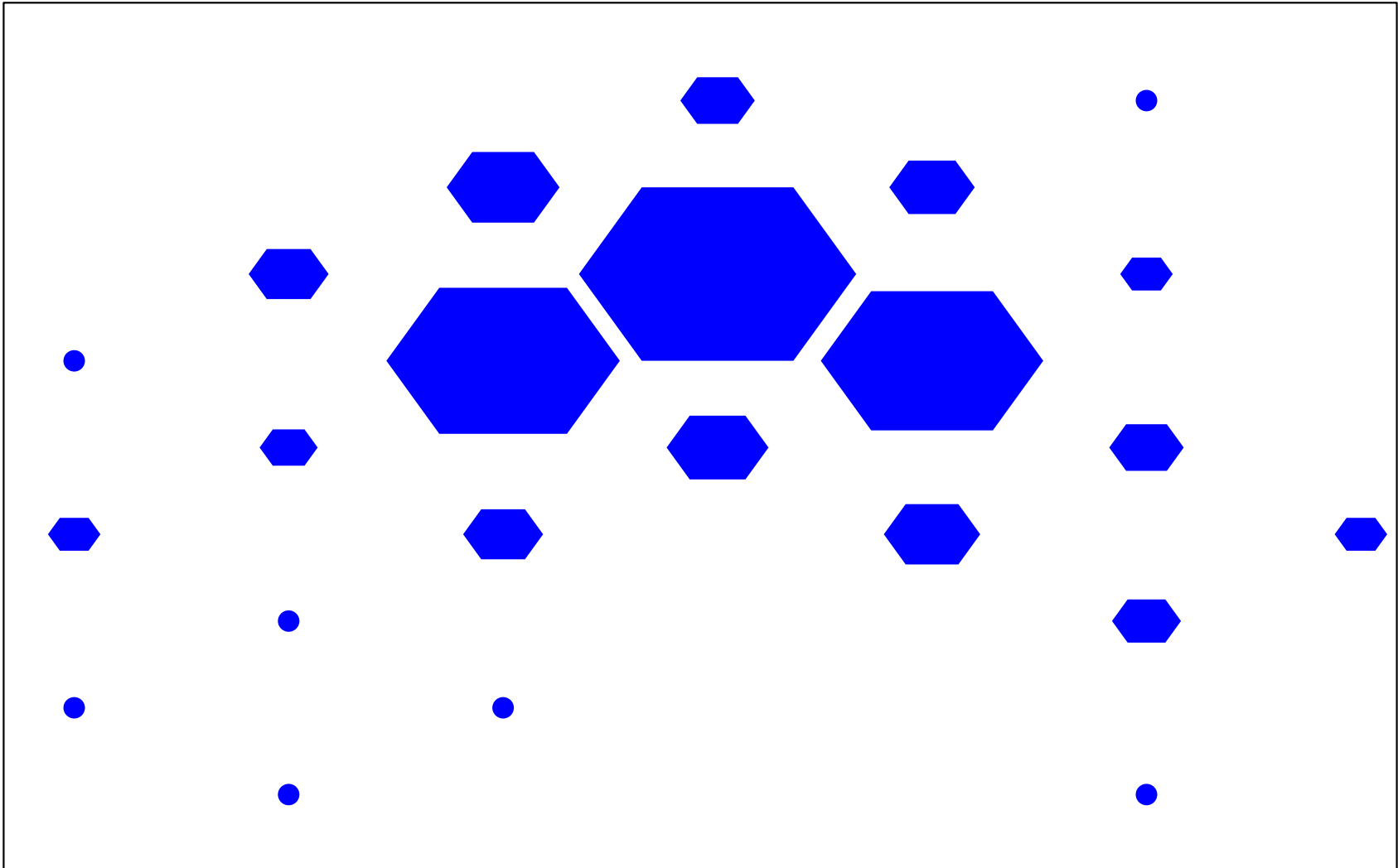
Binning in two dimensions;



# Hexbin – a better way

---

Binning in two dimensions;





# Hexbin – a better way

---

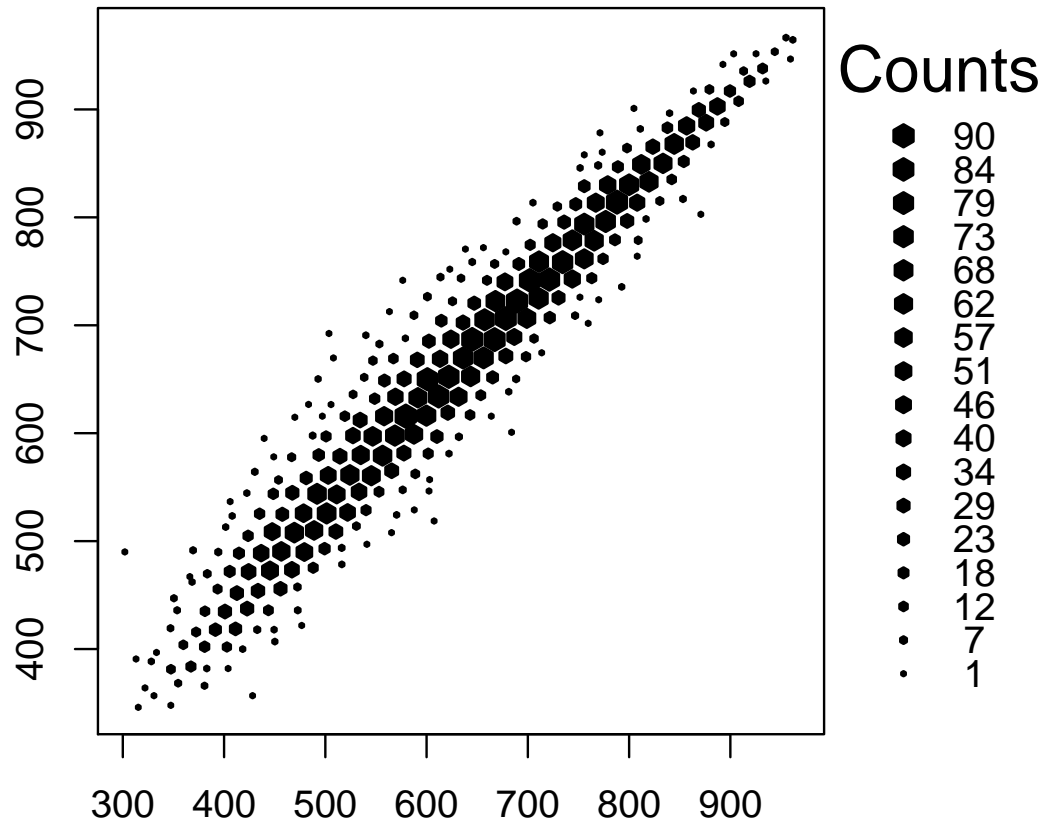
Back to schools

Now with `hexbin`; recall we download from Bioconductor, not CRAN

```
> biocLite("hexbin")  
> library(hexbin)  
> with(apipop, plot(hexbin(api99,api00), style="centroids"))
```

# Hexbin – a better way

---



# GWAS analysis

---

Genome-Wide Association Studies (GWAS) are currently popular – typically, these genotype e.g. 1M SNPs on several thousand subjects in (large) established studies

- Usually on 1000's of subjects
- 'Simple'  $t$ -tests, regressions, for each SNP (like microarrays)
- 1M *anything* takes a long time! (up to 72 hours)
- Just **loading** big datasets is non-trivial – but some tools are available

# GWAS analysis

---

`snpMatrix` is a Bioconductor package for GWAS analysis – maintained by David Clayton (analysis lead on Wellcome Trust)

```
biocLite("snpMatrix")  
library(snpMatrix)  
data(for.exercise)
```

A ‘little’ case-control dataset (Chr 10) based on HapMap – three objects; `snp.support`, `subject.support` and `snps.10`

# GWAS analysis

---

```
> summary(snp.support)
  chromosome    position      A1      A2
Min.      :10   Min.      : 101955  A:14019  C: 2349
1st Qu.   :10   1st Qu.   : 28981867  C:12166  G:12254
Median    :10   Median    : 67409719  G: 2316  T:13898
Mean      :10   Mean      : 66874497
3rd Qu.   :10   3rd Qu.   :101966491
Max.      :10   Max.      :135323432

> summary(subject.support)
      cc      stratum
Min.    :0.0   CEU      :494
1st Qu. :0.0   JPT+CHB :506
Median  :0.5
Mean    :0.5
3rd Qu. :1.0
Max.    :1.0
```

# GWAS analysis

---

```
> show(snps.10) # show() is generic
A snp.matrix with 1000 rows and 28501 columns
Row names:  jpt.869 ... ceu.464
Col names:  rs7909677 ... rs12218790
> summary(snps.10)
$rows
  Call.rate      Heterozygosity
Min.   :0.9879   Min.   :0.0000
Median :0.9900   Median :0.3078
Mean   :0.9900   Mean   :0.3074
Max.   :0.9919   Max.   :0.3386
$cols
  Calls      Call.rate      MAF      P.AA
Min.   : 975   Min.   :0.975   Min.   :0.0000   Min.   :0.00000
Median : 990   Median :0.990   Median :0.2315   Median :0.26876
Mean   : 990   Mean   :0.990   Mean   :0.2424   Mean   :0.34617
Max.   :1000   Max.   :1.000   Max.   :0.5000   Max.   :1.00000
  P.AB      P.BB      z.HWE
Min.   :0.0000   Min.   :0.00000   Min.   : -21.9725
Median :0.3198   Median :0.27492   Median :  -1.1910
Mean   :0.3074   Mean   :0.34647   Mean   :  -1.8610
Max.   :0.5504   Max.   :1.00000   Max.   :   3.7085
NA's   : 4.0000
```

# GWAS analysis

---

- 28501 SNPs, all with Allele 1, Allele 2
- 1000 subjects, 500 controls (`cc=0`) and 500 cases (`cc=1`)
- **Far too much** data for a regular `summary()` of `snps.10` – even in this small example

# GWAS analysis

---

We'll use just the column summaries, and a (mildly) 'clean' subset;

```
> snpsum <- col.summary(snps.10)
> use <- with(snpsum, MAF > 0.01 & z.HWE^2 < 200)
```

```
> table(use)
```

```
use
```

```
FALSE TRUE
```

```
317 28184
```



# GWAS analysis

---

Now do single-SNP tests for each SNP, and extract the  $p$ -value for each SNP, along with its location;

```
tests <- single.snp.tests(cc, data = subject.support,  
+ snp.data = snps.10)
```

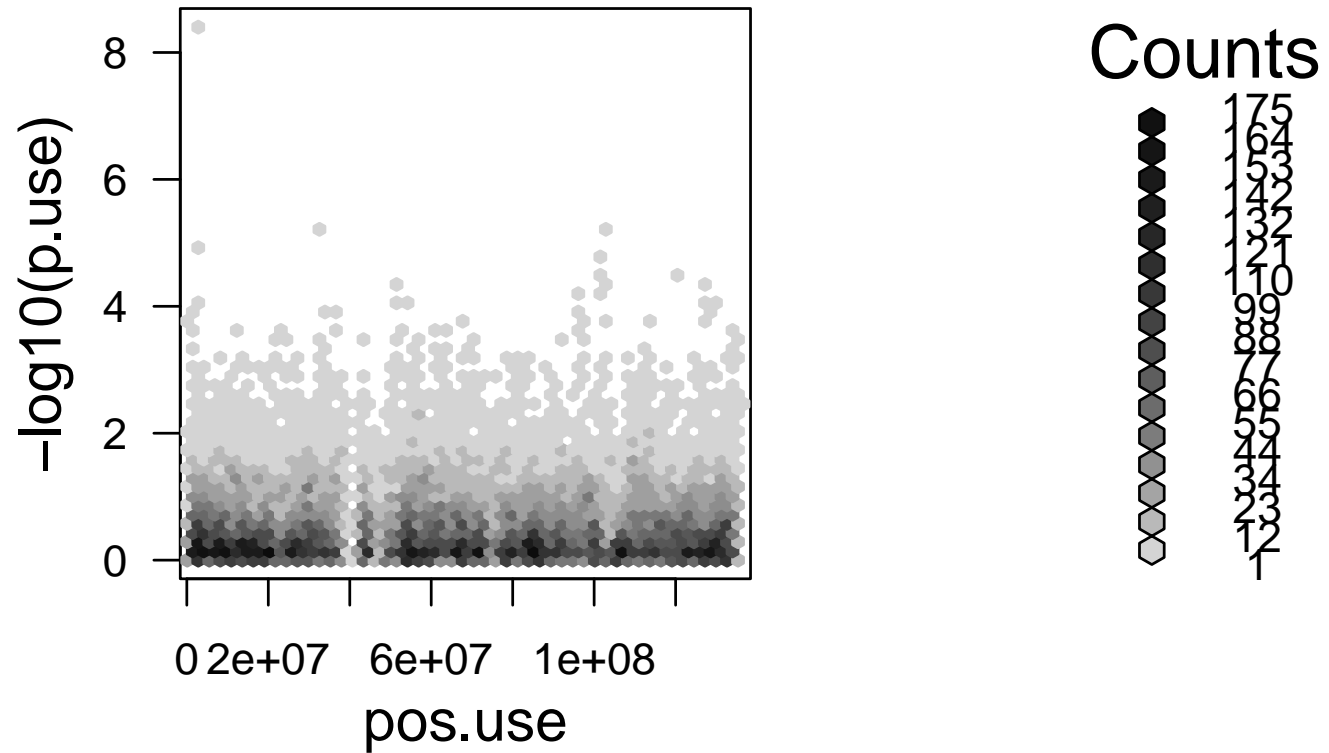
```
pos.use <- snp.support$position[use]  
p.use   <- p.value(tests, df=1)[use]
```

We'd usually give a table of 'top hits,' but...

# GWAS analysis

---

```
plot(hexbin(pos.use, -log10(p.use), xbin = 50))
```

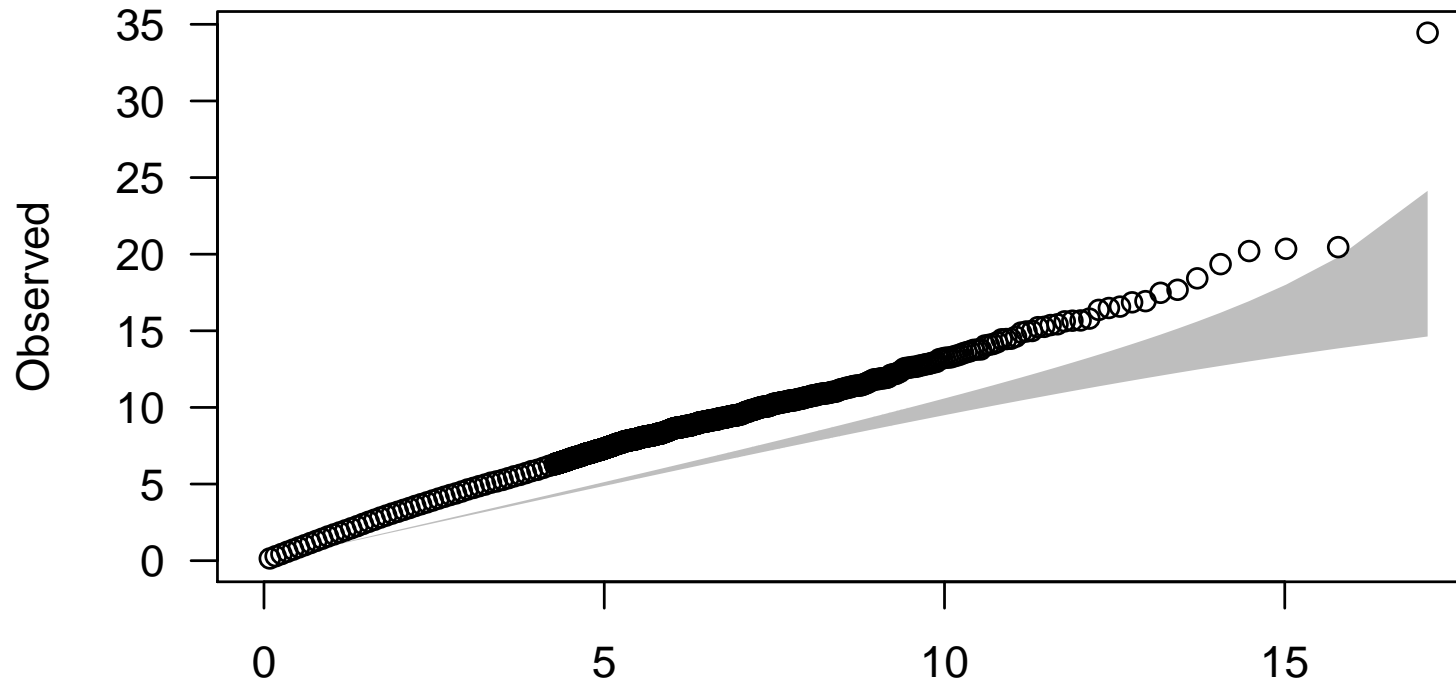


# GWAS analysis

---

```
qq.chisq(chi.squared(tests, df=1)[use], df=1)
```

QQ plot



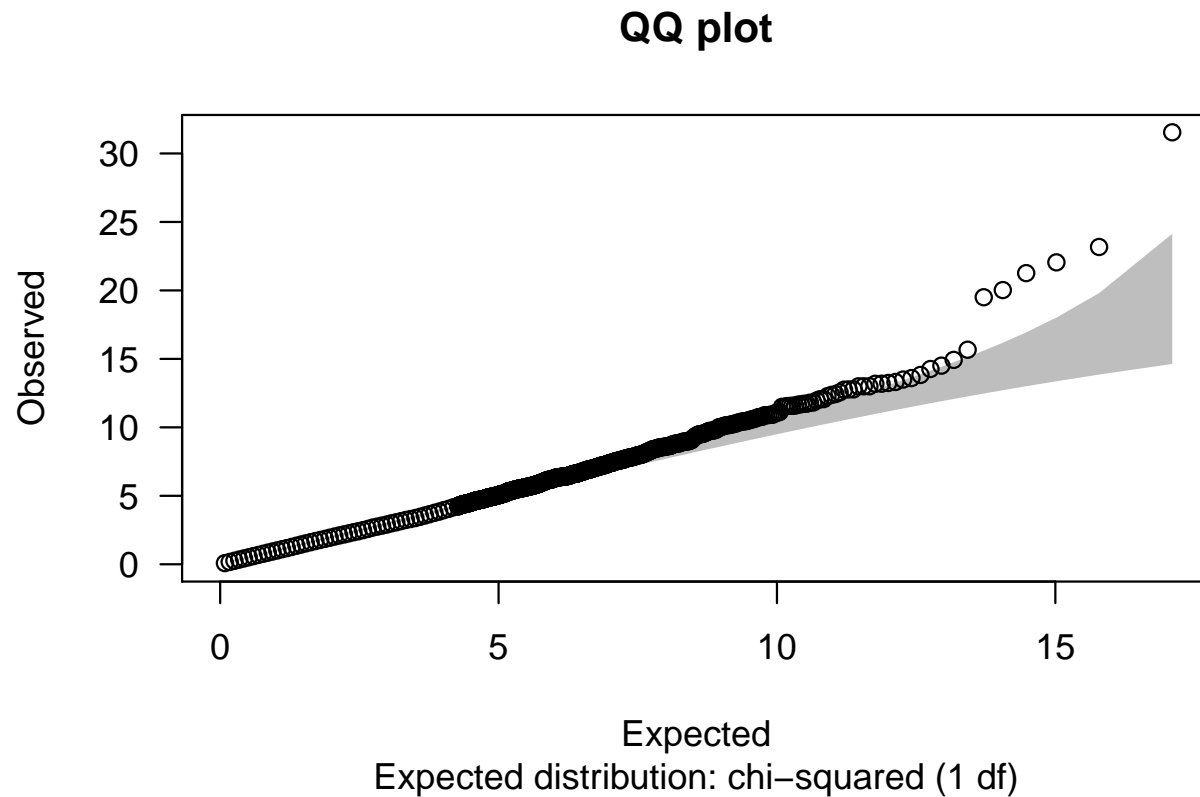
Expected

Expected distribution: chi-squared (1 df)

# GWAS analysis

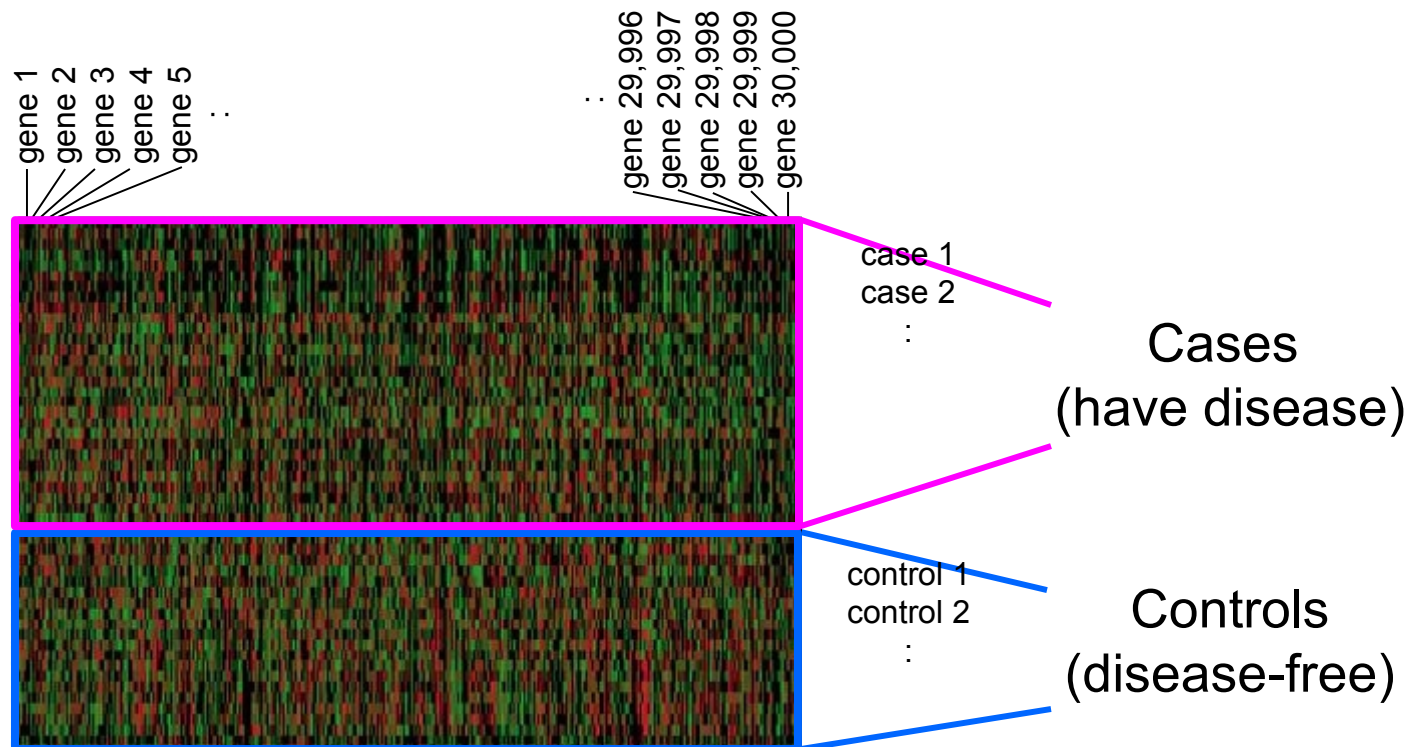
---

```
tests2 <- single.snp.tests(cc, stratum, data = subject.support,  
+ snp.data = snps.10)  
qq.chisq(chi.squared(tests2, 1)[use], 1)
```



# Significance Analysis of Microarrays (SAM)

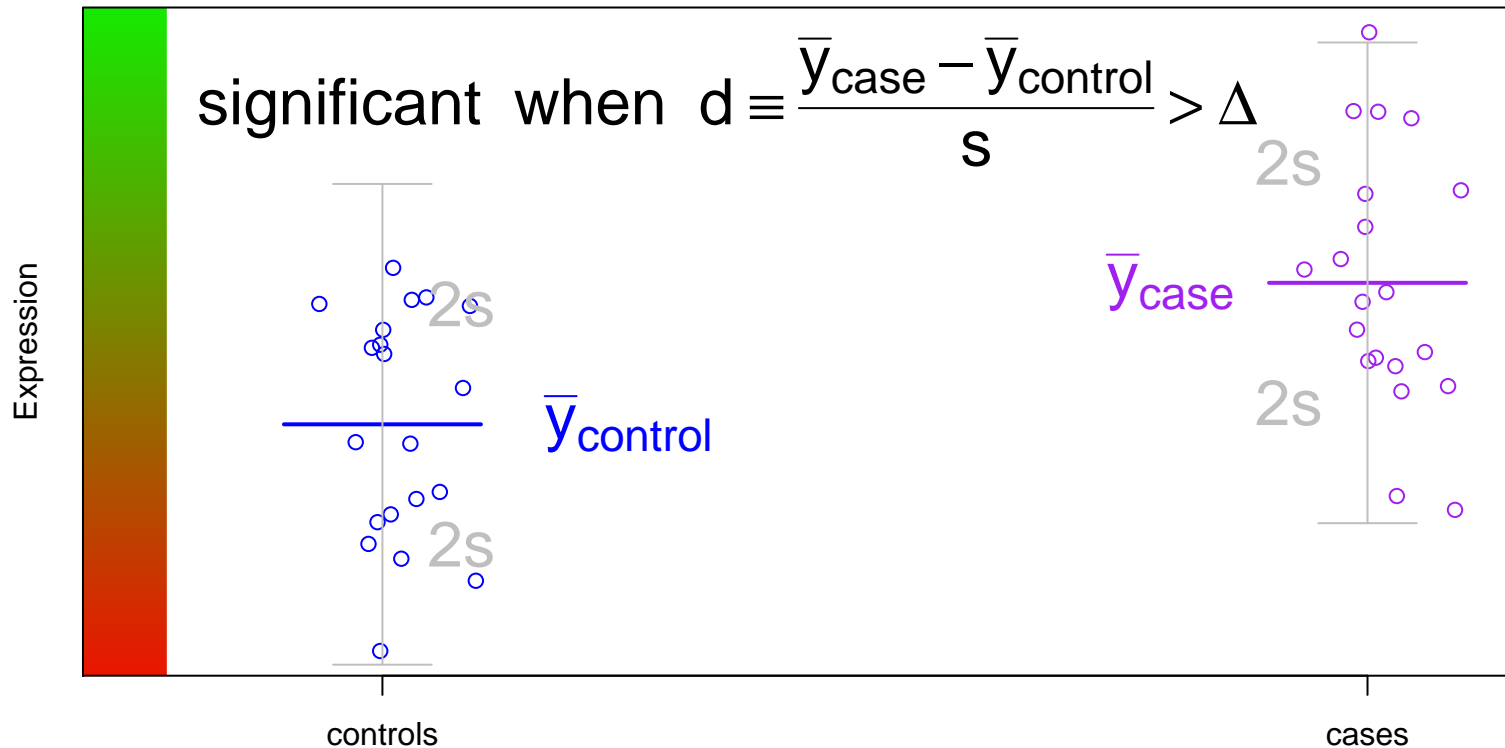
SAM is a popular new method (Tusher et al 2001) which identifies **differentially expressed genes**



i.e. large red/green difference between cases and controls

# Significance Analysis of Microarrays (SAM)

Why so popular? Here's the traditional method;



Do this  $\times 30,000$  genes;  $d$  in each is **quite unstable**. Small values of  $s$  give large  $d$ , which may give **false positive** results

# Significance Analysis of Microarrays (SAM)

---

SAM has a quick fix for this problem;

$$\begin{array}{ccc} \text{Traditional} & & \text{SAM} \\ d_i = \frac{\bar{y}_{i,\text{case}} - \bar{y}_{i,\text{control}}}{s_i} & d_i = & \frac{\bar{y}_{i,\text{case}} - \bar{y}_{i,\text{control}}}{s_i + s_0} \end{array}$$

For each gene (each  $i$ ), SAM's  $s_0$  **borrow strength** from the other genes.

SAM (and siggenes) then does some clever permutation testing to produce False Discovery Rates

# Significance Analysis of Microarrays

## (SAM)

---

Golub et al (1999) give differential expression for 3,051 genes, in 27 'controls' (*ALL*) and 11 'cases' (*AML*)

```
> library(multtest)
> data(golub)
> table(golub.cl)
  0  1
27 11
```

Now let's do the SAM analysis; we give a **random seed** for the permutations – and tell R how many to do;

```
> sam.out <- sam(golub, golub.cl, B=100, rand = 123)
```

... takes only a few seconds. Use B=1000 or more if you can



# Significance Analysis of Microarrays (SAM)

---

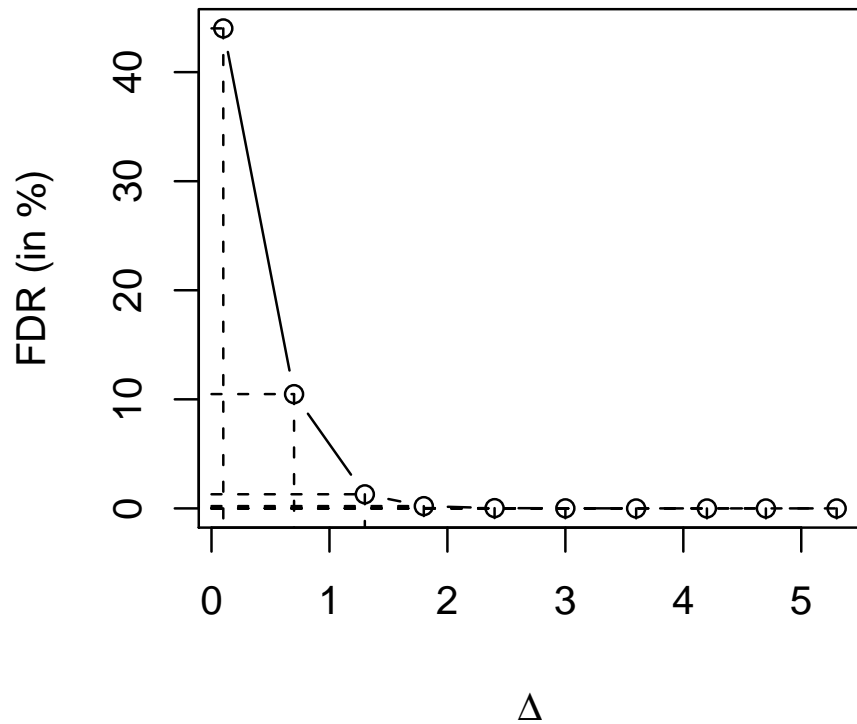
```
> summary(sam.out)
s0 = 0.0584 (The 0 % quantile of the s values.)
Number of permutations: 1000
  Delta  p0  False Called  FDR cutlow cutup  j2  j1
1  0.1 0.499 2420.329 2742 0.440123 -0.160 0.244 1446 1756
2  0.7 0.499 264.208 1257 0.104804 -1.247 1.438 746 2541
3  1.3 0.499 13.526 521 0.012945 -2.270 2.488 325 2856
4  1.8 0.499 0.903 215 0.002094 -3.119 3.311 139 2976
5  2.4 0.499 0.043 76 0.000282 -4.157 4.259 44 3020
6  3.0 0.499 0.003 15 9.97e-05 -5.577 5.139 4 3041
7  3.6 0.499 0 5 0 -Inf 5.971 0 3047
8  4.2 0.499 0 2 0 -Inf 7.965 0 3050
9  4.7 0.499 0 2 0 -Inf 7.965 0 3050
10 5.3 0.499 0 2 0 -Inf 7.965 0 3050
```

p0 is the **prior** probability of differential expression. Also note that the FDR values are **rounded**

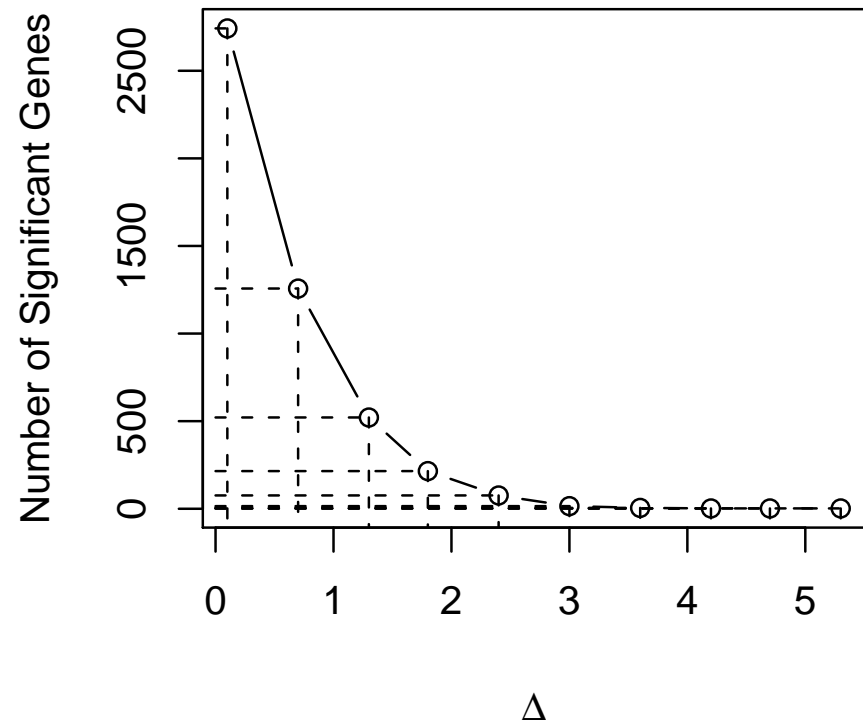
# Significance Analysis of Microarrays (SAM)

```
> plot(sam.out)
```

Delta vs. FDR

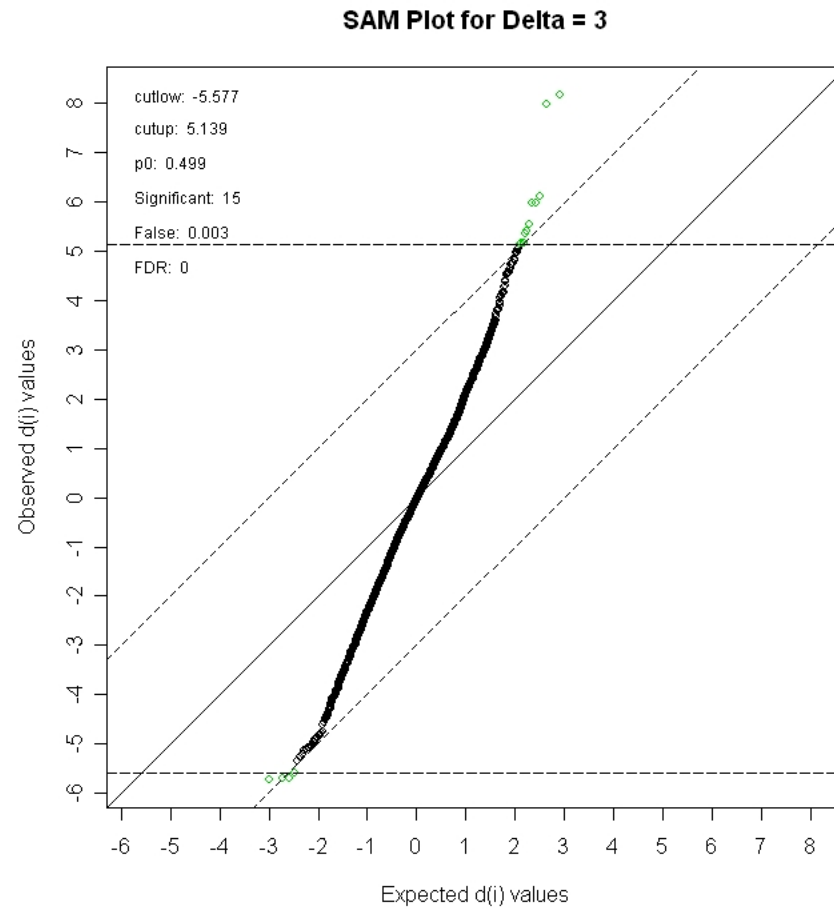


Delta vs. Significant Genes



# Significance Analysis of Microarrays (SAM)

```
> plot(sam.out, 3) #specifies Delta
```



# Microarray analysis with limma

---

The `limma` package can do **several** analyses for microarrays. It reads in **raw data**, in standard formats

```
> library(limma)
> my.files <- dir(pattern=".spot")
> my.files
[1] "swirl.1.spot" "swirl.2.spot" "swirl.3.spot" "swirl.4.spot"
> RG <- read.maimages(my.files, source="spot")
Read swirl.1.spot
Read swirl.2.spot
Read swirl.3.spot
Read swirl.4.spot
```

# Microarray analysis with limma

---

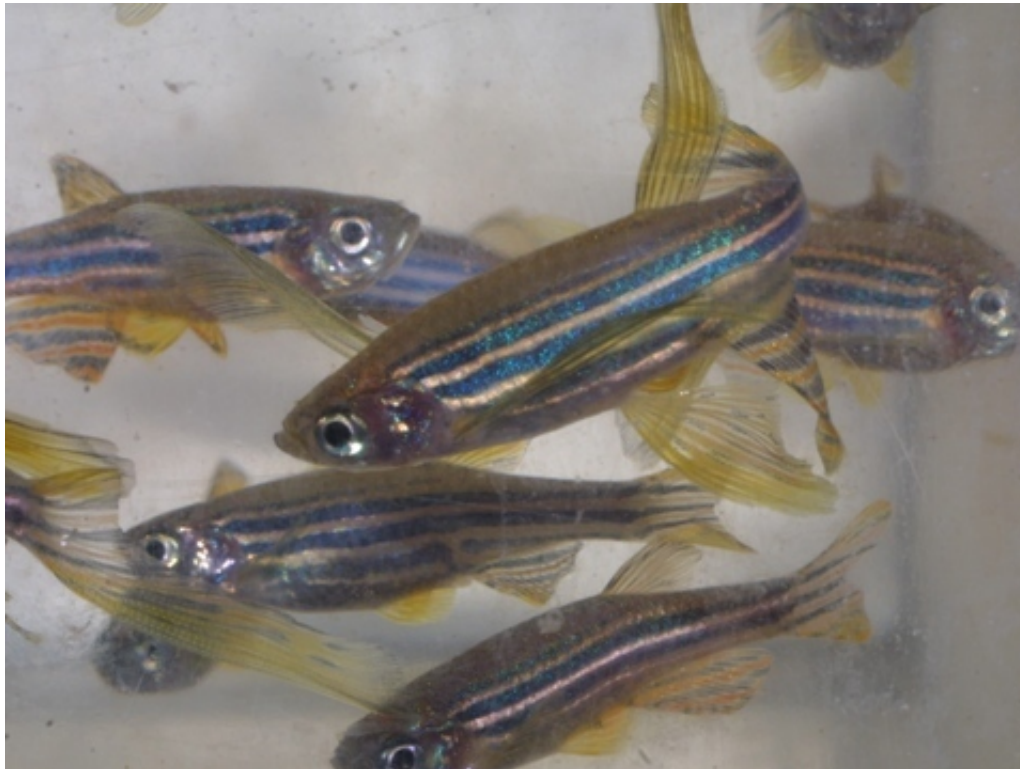
What is `swirl1`? A mutation affecting **zebrafish**



# Microarray analysis with limma

---

What is `swirl1`? A mutation affecting **zebrafish**

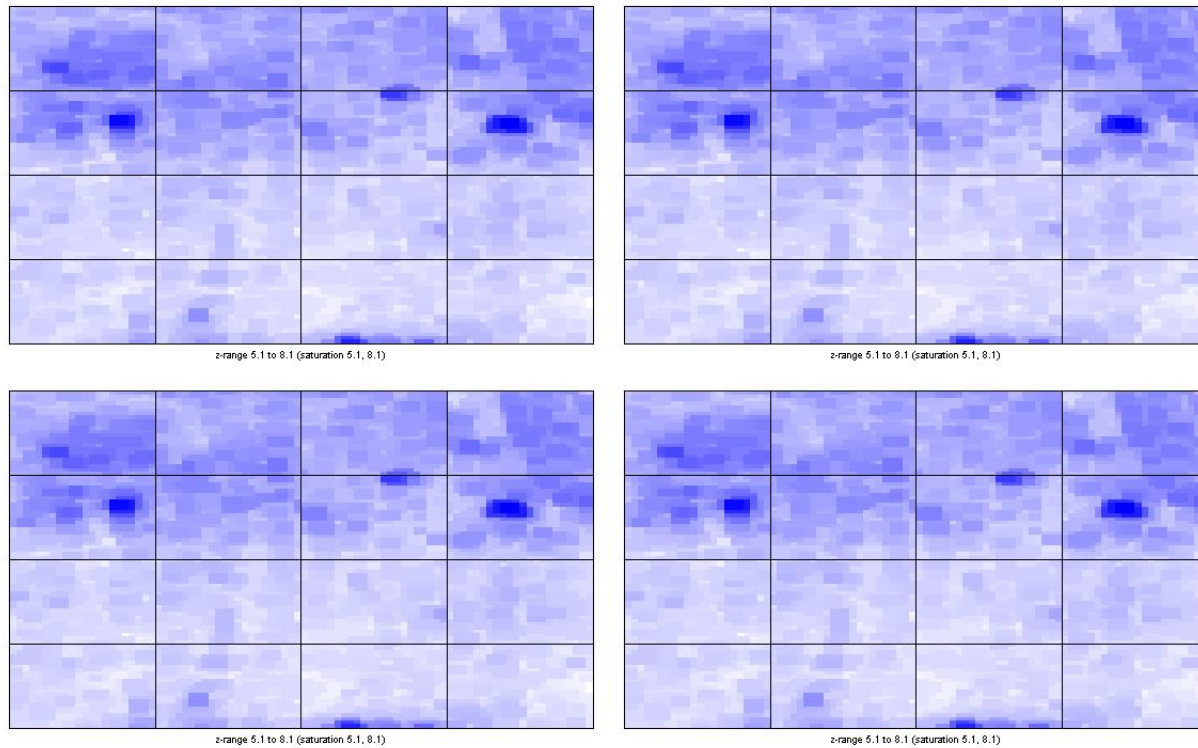


We have 2 mutants, and 2 wild-type fish

# Microarray analysis with `limma`

---

Here are the red intensities from each microarray;



– need to **normalize** each array (or get a bigger sample!)

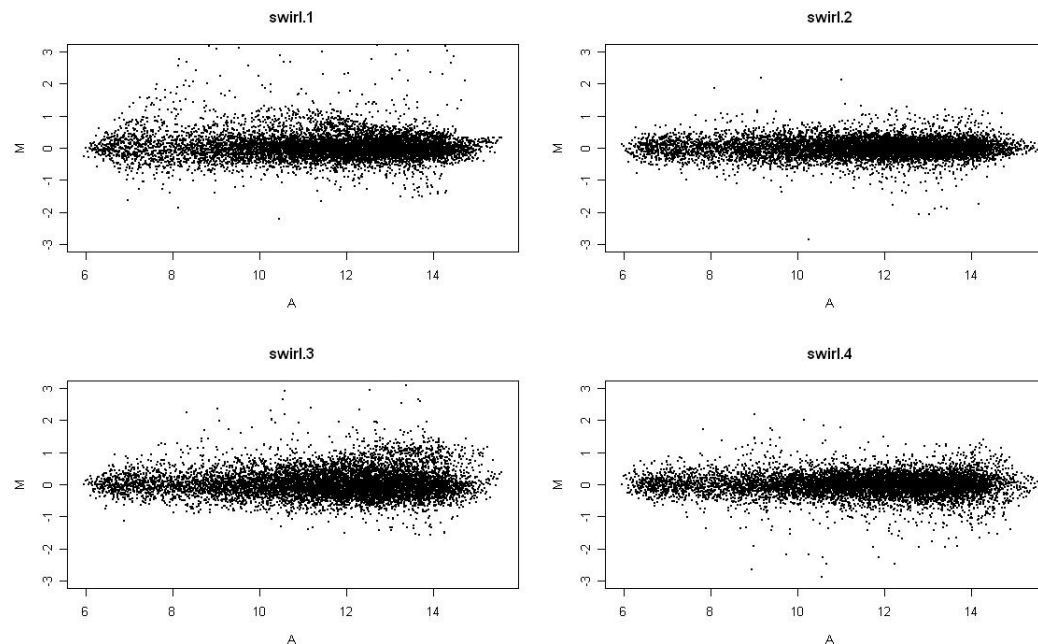
# Microarray analysis with limma

---

limma has 'default' normalization techniques

```
> MA1 <- normalizeWithinArrays(RG)
```

```
> MA2 <- normalizeBetweenArrays(MA1)
```



Can you guess where the 'signals' are?



# Microarray analysis with limma

---

limma fits 'plain' models to each gene, and also 'robustifies' them with an Empirical Bayes approach (much the same as SAM)

```
> fit1 <- lmFit(MA2, design=c(-1,1,-1,1))
> options(digits=3); toptable(fit, n=30, adjust="fdr")
```

	M	t	P.Value	adj.P.Val	B
2961	-2.66	-20.8	1.44e-07	0.00121	7.55
3723	-2.19	-17.6	4.59e-07	0.00194	6.75
1611	-2.19	-16.1	8.44e-07	0.00238	6.29
7649	-1.60	-14.2	2.02e-06	0.00326	5.58
515	1.26	13.7	2.55e-06	0.00326	5.39

```
> fit2 <- eBayes(fit1)
> options(digits=3); topTable(fit2, n=30, adjust="fdr")
```

	Block	Row	Column	ID	Name	M	A	t	P.Value	adj.P.Val	B
2961	6	14	9	fb85d05	18-F10	-2.66	10.33	-20.8	1.44e-07	0.00121	7.55
3723	8	2	3	control	Dlx3	-2.19	13.24	-17.6	4.59e-07	0.00194	6.75
1611	4	2	3	control	Dlx3	-2.19	13.45	-16.1	8.44e-07	0.00238	6.29
7649	15	11	17	fb58g10	11-L19	-1.60	13.49	-14.2	2.02e-06	0.00326	5.58
515	1	22	11	fc22a09	27-E17	1.26	13.19	13.7	2.55e-06	0.00326	5.39